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Abstracts of

# Role of Humoral Factors in the Progression of HIV Disease

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# THE ROLE OF CYTOKINES IN IMMUNE RESPONSE TO HIV

Mario Clerici[1], Maria Luisa Villa[1] and Gene M. Shearer[2]
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The mechanisms that regulate induction of distinct immune responses to different pathogens have recently been clarified by the identification in mice and man of two functionally distinct compartments defined as "T helper 1" (TH-1) and "T helper 2" (TH-2). The recognition and definition of these two subsets has been based on clonal isolates of purified CD4+ T lymphocytes. Functionally, TH-1 responses are characterized by secretion of IFN-$\gamma$ and interleukin-2 (IL-2) and subsequent promotion of cell mediated immunity (CMI); TH-2 responses are characterized by secretion of interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), and interleukin-10 (IL-10), with subsequent activation of humoral immunity and generation of antibodies. The induction of a TH-1 response induces the suppression of a TH-2 response, and vice versa. This trend toward reciprocity is mediated through the secretion of cross-regulatory cytokines (IFN-$\gamma$ suppresses TH-2 responses; IL-4 and IL-10 suppress TH-1 responses). These two compartments are differentially responsible for immune defense, and the preferential activation of TH-1 or TH-2 responses to agents that provoke disease through different mechanisms optimizes the immune response. Thus, the activation of a TH-1 response appears to be more efficient in immune defense against intracellular pathogens and parasites, while TH-2 responses

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may be more protective against bacterial infections and toxins. We have studied cytokine production profiles in HIV infection by *in vitro* stimulation of whole peripheral blood mononuclear cells (PBMC) of HIV seropositive (HIV+) individuals and we have observed that HIV infection is characterized by a switch from a TH-1 to a TH-2 cytokine production profile. Nevertheless, because our observations are based on cytokine production by whole PBMC and not on clonal isolation, we prefer not to use the clonally defined TH-1 and TH-2 terminology, but rather to identify the cytokine patterns observed as type 1, characterized by high IL-2, high IFN-γ and low IL-4, low IL-10 (CMI-inducing) and type 2, characterized by low IL-2, low IFN-γ and high IL-4, high IL-10 (humoral immunity-inducing).

A number of recent reports has led to the hypothesis that CMI, driven by the production of type 1 cytokines, might be associated with augmented resistance to human immunodeficiency virus (HIV) infection in HIV seronegative (HIV-) individuals exposed to HIV, and with lack of progression to AIDS in HIV+ individuals. Conversely, seroconversion and fast progression to AIDS would be associated with a prevalence of humoral immunity over CMI, a condition characterized by low production of IFN-γ and IL-2, and a high production of IL-4, IL-6 and IL-10.

To first summarize the observations that suggest how type 1 response may be associated with lack of progression to AIDS in HIV+ individuals: 1) *in vitro* antigen and mitogen-stimulated IL-2 production by peripheral blood mononuclear cells (PBMC) of HIV+ individuals progressively declines after HIV infection; this decline is accompanied by sharp increases in

demonstrated by the observation that IL-4 knock-out mice do not develop MAIDS upon infection with LP-BM5.

A second line of evidences suggest that type 1 responses may be associated with augmented resistance to HIV infection in HIV- individuals. To summarize: 1) exposure to HIV in the absence of seroconversion is associated with the exclusive presence of HIV-specific T-lymphocytes, characterized as IL-2 producing cells. HIV-specific, IL-2 producing lymphocytes have been detected in many individuals that do not seroconvert despite their belonging to groups at risk for HIV infection. These at-risk groups include: gay men, intravenous drugs users, sexual partners of HIV+ individuals, health care workers exposed to HIV through needle sticks, and newborns infants of $HIV^+$ mothers. 2) HIV infection in newborn of HIV+ mothers is associated with absence of HIV specific IL-2 producing T lymphocytes. 3) CD8+ HLA class I restricted HIV-specific cytotoxic T lymphocytes are detectable in PBMC of HIV- individuals at risk for HIV infection. 4) Intrarectal exposure of macaques to different doses of simian immunodeficiency virus (SIV) induces seroconversion, simian AIDS and death only in the macaques exposed to high doses of SIV. SIV-specific immune responses in these macaques are characterized by high titers of SIV specific antibodies and weak or absent SIV-specific T lymphocytes. In contrast, macaques exposed to low doses of SIV do not seroconvert and show potent SIV-specific T cell responses. Additionally, these macaques appear to be protected upon challenge with high doses of SIV. 5) In a preliminary report, when PBMC from volunteers immunized with HIV candidate vaccines are used to reconstitute the deficient immune system of SCID mice, the mice

mitogen-stimulated production of IL-4 and IL-10.The switch from a type 1 to a type 2 cytokine production pattern in HIV infection has been analyzed and confirmed at different levels: cytokine-specific mRNA by PCR analysis, production of cytokines by ELISA, and very recently, clonal isolation. 2) Defective IL-2 production by HIV+ PBMC can be circumvented in vitro by stimulation of such PBMC in the presence of neutralizing antibodies directed toward IL-4 and/or IL-10. 3) The presence and the magnitude of IL-2 production defects in HIV+ asymptomatic individuals is predictive for the rate of decline in CD4 counts, time to development of AIDS, and time to death. 4) Preliminary data show that the pattern of cytokine production by PBMC of HIV+ long term survivors is that of a type 1 response (high IL-2 and IFN-γ, low IL-4 and IL-10). 5) Clonal isolates from HIV+ patients enrolled in longitudinal studies are initially characterized as IL-2 and IFN-γ producing clones, and subsequently as IL-4 producing clones. 6) TH-1 and TH-2 T lymphocytes clones are differentially susceptible to HIV infection as HIV replicates only inside TH-2 clones.7) TH-1 but not TH-2 T lymphocytes clones are able to prevent *in vitro* HIV infection of autologous and HLA mismatched cells. Additional evidence supporting the hypothesis of a type 1-to-type 2 cytokine profile switch in HIV infection is offered by a murine model in which susceptible strains of mice infected with LP-BM5 murine leukemia virus develop an immunodeficiency syndrome defined murine AIDS (MAIDS). MAIDS is characterized by profound defects in the production of type 1 cytokines, accompanied by massive increases in the production of IL-4, IL-5, IL-6 and IL-10. That this switch is essential in the pathogenesis of MAIDS is

are protected from challenged with HIV only if the PBMC are obtained from volunteers in whom the vaccine induced generation of HIV-specific T lymphocytes.

Thus, it seem possible that type 1 responses are more important than type 2 responses for successfully impeding infection by HIV and SIV, and may be responsible for the long asymptomatic period that follows HIV and SIV infection. These observations suggest the possibility of using cytokines based therapies in HIV infection with the double possible objective of preventing a type 1-to-type 2 switch and of restoring a type 1 profile in those patients in which HIV infection has already provoked alterations in the cytokine production profile. A possible candidate for cytokine based therapy of HIV infection is interleukin 12 (IL-12). This newly described lymphokine has several powerful functional properties, most interestingly its ability to alter the T cell cytokine balance in favor of type 1 cytokines with subsequent enhancement of CMI. Therefore, even if IL-12 cannot be defined as a TH-1 cytokine because it is not produced by T lymphocytes, IL-12 is a type 1 cytokine. As predicted by the type-1-to-type-2 cytokine production switch in HIV infection, IL-12 production is severely defective in HIV+ individuals when compared to HIV- controls. We recently described the ability of IL-12 to restore defective in vitro antigen-stimulated T cell proliferation and IL-2 production, and defective mitogen-stimulated IFN-γ generation in HIV+ individuals. Furthermore, preliminary data suggest that IL-12 is capable of preventing the development of MAIDS in susceptible mice infected with LP-BM5.

Finally, cytokines based therapy of HIV infection could theoretically be based upon three different approaches: 1) use of type 1 cytokines (IFN-$\gamma$; IL-2; IL-12); 2) use of anti-type 2 neutralizing antibodies (anti-IL-4; anti-IL-10); and 3) use of soluble type 2 cytokines receptors (soluble IL-4 receptor). The clinical feasibility and the therapeutical usefulness of each one of these approaches will hopefully be soon evaluated in controlled clinical trials.

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# AUTOANTIBODIES AGAINST CD4+ LYMPHOCYTES IN HIV-INFECTED HEMOPHILIA PATIENTS

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The pathogenesis of the CD4+ lymphocyte depletion in HIV-infected patients is poorly understood. The direct cytotoxic effect of HIV on the CD4+ lymphocytes, the capability of HIV-infected cells to form syncytia and the hyperglutamataemia of the patients do not explain this phenomenon completely. Autoimmune mechanisms, especially autoreactive T lymphocytes and autoantibodies gain increasing relevance for the conception of the AIDS pathogenesis. In this review we report on our autoantibody studies.

Autoantibodies may be cell-depleting or, alternatively, immunoregulatory. Depleting autoantibodies have their targets on the surface of the CD4+ lymphocytes. Regulatory autoantibodies, however, are antiidiotypic and react with other components of the immune network that have itself regulating or effector function. We studied both types of autoantibodies in HIV-infected hemophilia patients.

The major target structure on CD4+ lymphocytes for autoantibodies should be the CD4 receptor itself. Recombinant CD4 serves as a model for the native CD4 receptor. Antibodies against recombinant CD4 are found in 22% of the AIDS/ARC patients, 10% of the asymptomatic HIV+ and 17% of the HIV-hemophilia patients but only in 1% of the healthy controls tested in parallel **(1)**. Antibodies against recombinant CD4 purified by affinity chromatography do not react with CD4+ lymphocytes of healthy individuals. Because these antibodies do not recognize native CD4 it is not surprising that the occurrence of antibodies against recombinant CD4 is not associated with the CD4+ cell counts in the blood of the patients **(1)**. Another target structure on CD4+ lymphocytes are MHC class II antigens. Activated CD4+ lymphocytes express MHC class II antigens on the surface. The majority of our HIV-infected hemophilia patients

have cold reactive B lymphocyte antibodies with postulated MHC class II antigen specificity (2).

Antibodies that coat CD4+ lymphocytes in-vivo may be of preferential relevance for the AIDS pathogenesis. With flowcytometry we are able to detect immunoglobulin covered CD4+ lymphocytes in the blood of the patients (3). We characterized the isotype and the complement activation capacity of the autoantibodies and found diverse autoantibody patterns on CD4+ lymphocytes. The diverse autoantibody patterns were associated with the CD4+ cell counts in the blood and seem to be typical for the different clinical stages of the disease. At the beginning of the disease IgM autoantibodies were found on CD4+ lymphocytes. When additional complement-fixing autoantibodies were detectable on the CD4+ lymphocytes, the CD4+ cell count decreased further on. Finally, at very low CD4+ cell counts of $< 100$/ul, attached gp120 is demonstrable on the CD4+ cells (4). Obviously, the immune-complex covered CD4+ cells are reduced by phagocytosis, antibody-dependent cellular cytotoxicity, cytotoxic T lymphocytes or antibody dependent complement dependent lysis.

The immune-complex load of the CD4+ lymphocytes induces also impaired T lymphocyte function. Patients with immunoglobulin coated CD4+ lymphocytes in the blood showed an impaired T helper cell function for the T cell dependent B lymphocyte response in vitro (5). When soluble gp120 is found on the CD4+ lymphocytes the global mitogenic and allogeneic response of the lymphocytes in-vitro decreases with the growing immune-complex load of the CD4+ lymphocytes (6). As a consequence of the T lymphocyte dysfunction, the monocyte/macrophage activity in-vivo increases and the serum neopterin levels raise (6).

Beside the autoantibodies detectable on CD4+ lymphocytes other autoantibody types in the serum that are both crossreactive with epitopes on CD4+ lymphocytes and reactive with other immunoglobulins or antiidiotypic components of the immune network seem to have a role in the AIDS pathogenesis. These antibodies may be both depletion-inducing and immuno-regulatory. For example, antibodies directed against the Fab part of immunoglobulins can have both qualities.

The titers of IgG-anti-Fab and IgA-anti-Fab autoantibodies were associated with the clinical course of the patients. Both IgG-anti-

Fab and IgA-anti-Fab autoantibodies were significantly correlated with the CD4+ lymphocyte counts of the patients. The IgA-anti-Fab autoantibodies were also associated with the serum neopterin levels **(7-10)**.

Anti-Fab antibodies may influence the CD4+ cell counts by binding to CD4+ lymphocytes. Anti-Fab antibodies from HIV+ sera purified by affinity chromatography reacted with CD4+ lymphocytes of healthy individuals that were preincubated with recombinant gp120 **(9)**. Patients with high IgG-anti-F(ab')$_{2\gamma}$ antibody titers showed both high titers of anti-gp120 antibodies and high titers of antibodies directed against the CH1 domain of IgG **(10)**. Obviously, these autoantibodies that are primarily directed against the CH1 domain are induced by circulating gp120. It seems that they are able to intensify the depletion of the CD4+ lymphocytes by binding to gp120 coated CD4+ lymphocytes.

Conceivably, the anti-Fab antibodies regulate the immune-system by interaction with other antibodies and components of the immune network. Sequential studies of HIV+ patients showed similar titers of anti-anti-gp120 and anti-recombinant-CD4 antibodies. When HIV+ patients were grouped according to their CD4+ lymphocyte counts in the blood, the IgG-anti-anti-gp120 titers of the different groups paralleled the IgG-anti-anti-CD8 and anti-recombinant-CD4 antibody titers whereas IgG-anti-Fab titers showed an opposite behaviour **(11)**. It is known that Fab binds recombinant CD4, CD8 binds MHC I, and CD4 binds MHC II and gp120. Based on these interactions, the existence of two biologically distinct groups of molecules with similarities to MHC I (anti-Fab, anti-CD8, CD4) or MHC II (gp120, Fab, anti-anti-gp120, anti-anti-CD8, anti-recombinant-CD4, CD8) can be hypothesized. Disturbances of the equilibrium between group I and group II autoantibodies appears to play a role in the progression of the disease.

Our data support the hypothesis that autoimmune mechanisms contribute to the CD4+ lymphocyte dysfunctions and to the CD4+ lymphocyte depletion in HIV-infected individuals. The autoimmune mechanisms are triggered by HIV and in part by soluble HIV proteins, especially gp120.

## References


(1) Daniel V, Weimer R, Zettlmeissl G, Langner K, Zimmermann R, Opelz G: Autoantibodies in HIV infected hemophilia patients against different epitopes on CD4+ lymphocytes and recombinant CD4. Vox Sang 1992; 62:39-44

(2) Daniel V, Schimpf K, Opelz G: Lymphocyte autoantibodies and alloantibodies in HIV-positive haemophilia patients. Clin Exp Immunol 1989; 75:178-83

(3) Daniel V, Weimer R, Schimpf K, Opelz G: Autoantibodies against CD4- and CD8-positive T lymphocytes in HIV-infected hemophilia patients. Vox Sang 1989; 57:172-6

(4) Daniel V, Süsal C, Prodeus AP, Weimer R, Zimmermann R, Huth-Kühne A, Opelz G: CD4+ lymphocyte depletion in HIV infected patients is associated with gp120-immunoglobulin-complement attachment to CD4+ cells. Vox Sang 1993; 64:31-36

(5) Weimer R, Daniel V, Zimmermann R, Schimpf K, Opelz G: Autoantibodies against CD4+ cells are associated with CD4 helper defects in human immunodeficiency virus-infected patients. Blood 1991; 77:133-40

(6) Daniel V, Süsal C, Weimer R, Zimmermann R, Huth-Kühne A, Opelz G: Association of T cell and macrophage dysfunction with surface gp120-immunoglobulin-complement complexes in HIV-infected patients. Clin Exp Immunol 1993; 93:152-56

(7) Süsal C, Daniel V, Oberg HH, Terness P, Huth-Kühne A, Zimmermann R, Opelz G: Striking inverse association of IgG-anti-Fab$_{\gamma}$ antibodies and CD4 cell counts in patients with acquired immunodeficiency syndrome (AIDS)/ AIDS-Related Complex. Blood 1992; 79:954-7

(8) Süsal C, Daniel V, Doerr C, Zimmermann R, Huth-Kühne A, Opelz G: IgA-anti-Fab autoantibodies and disease progression in AIDS. Immunol Lett 1993; 36:27-30

(9) Süsal C, Oberg HH, Daniel V, Dörr C, Terness P, Huth-Kühne A, Zimmermann R, Opelz G: Isotypes and IgG subclasses of anti-Fab antibodies in human immunodeficiency virus-infected hemophilia patients. Vox Sang 1994; 66:37-45

**(10)** Süsal C, Lewin IV, Stanworth DR, Terness P, Daniel V, Oberg HH, Huth-Kühne A, Zimmermann R, Opelz G: Anti-IgG autoantibodies in HIV-infected hemophilia patients. Vox Sang 1992; 62:224-229

**(11)** Süsal C, Hoffman GW, Daniel V, Grant M, Opelz G: Complementarities and network interactions in AIDS. J Autoimmunity 1993; 6:601-10

**(12)** Süsal C, Kröpelin M, Daniel V, Opelz G: Molecular mimicry between HIV-1 and antigen receptor molecules: a clue to the pathogenesis of AIDS. Vox Sang 1993; 65:10-17

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# THE ROLE OF A CYSTEINE AND GLUTATHIONE DEFICIENCY IN THE IMMUNOPATHOLOGY OF HIV INFECTION

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A large body of evidence from clinical studies and complementary laboratory experiments indicates that the acquired immunodeficiency syndrome (AIDS) may be the consequence of a virus-induced cysteine and glutathione (GSH) deficiency (1-3). HIV-infected persons of all stages of the disease have, on the average, markedly decreased plasma cystine and cysteine concentrations, decreased intracellular glutathione and elevated plasma glutamate levels (4-6). Elevated extracellular glutamate levels aggravate the cysteine deficiency, since glutamate inhibits competitively the membrane transport of cystine (7,8). Lymphocyte functions *in vitro* are augmented even by moderate elevations of extracellular cysteine and inhibited by elevation of the extracellular glutamate concentration. A significant correlation between individual $CD4^+$ T cell numbers and individual cystine and glutamate levels has also been found in a cohort of HIV-infected persons, in healthy human blood donors and in chimpanzees (6,9). $CD8^+$ T cells showed no significant correlation. A rapid and significant decrease of plasma cysteine levels, intracellular glutathione levels and an increase of plasma glutamate levels was also found in rhesus macaques 2 weeks after infection with the closely related $SIV_{mac251}$, but not in HIV-infected chimpanzees or $SIV_{agm}$-infected African green monkeys (9,10). The latter two species do not develop AIDS-like symptoms. Elevated plasma glutamate levels were found to be negatively correlated with lymphocyte functions and survival time also in patients with advanced malignancies (6,11,12).

One important consequence of a cysteine deficiency is the decrease of intracellular glutathione and glutathione disulfide levels. There is now a wealth of experimental evidence indicating that the T cell system is profoundly influenced even by relatively moderate changes of the extracellular cysteine concentration and the intracellular glutathione and glutathione disulfide levels. The limiting role of cysteine is mainly the consequence of the weak cystine transport activity of lymphocytes and the relatively low extracellular concentrations of reduced cysteine in blood plasma or cell cultures (5,8,13). *In vivo*, this limited and well regulated supply of cysteine may be required mainly in order to ensure a well balanced intracellular GSSG level that allows the optimal activation of

immunologically important NFκB-dependent genes (14-16). Suboptimal GSSG levels constrain the activation of nuclear translocation of the transcription factor NFκB, while superoptimal levels of GSSG constrain its transactivating potential by inhibiting its DNA binding activity. In healthy human subjects, the cysteine supply and intracellular glutathione levels of lymphocytes appear to be adjusted to the optimum, since mean and median glutathione levels are associated with maximum T cell numbers (17). Even a moderate decrease or increase of intracellular glutathione levels is associated with a detectable decrease of $CD4^+$ T cell numbers in seemingly healthy human subjects (17). It is, therefore, not surprising that the strongly decreased intracellular glutathione levels in HIV-infected patients are associated with a marked depletion of the $CD4^+$ T cell pool.

The more detailed analysis has finally shown that different immunological functions have clearly distinct requirements for cysteine and glutathione and that T cell responses against a strong mitogenic stimulus have a stronger requirement for cysteine and glutathione against a weak stimulus (8,18-24). This leads to the prediction that T cells that are constantly stimulated by environmental pathogens or superantigens may be more strongly affected by the glutathione deficiency in HIV-infected patients than T cells of other specificities.

Finally, we have shown that activated macrophages have a strong capacity to take up cystine, to release reduced cysteine into the extracellular space and to increase thereby the intracellular glutathione levels of activated T lymphocytes in their vicinity (7,18). This "cysteine pumping function" of the macrophages is expectedly strongly inhibited by elevated extracellular glutamate levels which competitively inhibit the membrane transport of cystine (7). The elevated plasma glutamate levels in HIV infected patients are therefore expected to inhibit also certain important macrophage functions.

In view of the established cysteine and glutathione deficiency in HIV infected persons, we have proposed to consider N-acetyl-cysteine for the treatment of these patients (1,25). NAC is a well established and safe drug with well documented toxicology and pharmacokinetics. A placebo controled study on healthy human subjects revealed that persons who moved during a 4-week observation period from the optimal to the suboptimal range of intracellular glutathione levels experienced, on the average, a 30% decrease of $CD4^+$ T cell numbers. This decrease was prevented by treatment with N-acetyl-cysteine (NAC) (17). Importantly, NAC was found to cause a relative increase of $CD4^+$ T cell numbers in spite of decreasing glutathione levels and

not by increasing the glutathione level. Taken together, our studies suggested that the immune system may be exquisitely sensitive not only against a cysteine and glutathione deficiency but also against an excess of cysteine.

References

1. W. Dröge, H.-P. Eck, and S. Mihm. HIV-induced cysteine deficiency and T-cell dysfunction - a rationale for treatment with N-acetyl-cysteine. Immunol. Today 13:211-214 (1992).
2. W. Dröge, H.-P. Eck, and S. Mihm. Cysteine and glutathione deficiency in HIV-infected persons and putative consequences for the expression of lymphokine receptor and cytokine genes. Medical Immunology 26:357-362 (1993).
3. W. Dröge, H.-P. Eck, S. Mihm, and D. Galter. Abnormal redox regulation in HIV infection and other immunodeficiency diseases. In: Oxidative Stress, Cell Activation and Viral Infection. C. Pasquier et al. (eds), Birkhäuser Verlag Basel/Switzerland, pp. 285-299 (1994).
4. W. Dröge, H.-P. Eck, H. Näher, U. Pekar, and V. Daniel. Abnormal amino acid concentrations in the blood of patients with acquired immune deficiency syndrome (AIDS) may contribute to the immunological defect. Biol. Chem. Hoppe-Seyler 369:143 (1988).
5. H.-P. Eck, H. Gmünder, M. Hartmann, D. Petzoldt, V. Daniel, and W. Dröge. Low concentrations of acid-soluble thiol (cysteine) in the blood plasma of HIV-1 infected patients. Biol. Chem. Hoppe-Seyler 370:101 (1989).
6. H.-P. Eck, T. Mertens, H. Rosokat, G. Fätkenheuer, C. Pohl, M. Schrappe, V. Daniel, H. Näher, D. Petzoldt, P. Drings, and W. Dröge. T4+ cell numbers are correlated with plasma glutamate and cystine levels: association of hyperglutamataemia with immunodeficiency in diseases with different etiologies. Int. Immunol. 4:7-13 (1992).
7. H.-P. Eck and W. Dröge. Influence of the extracellular glutamate concentration on the intracellular cyst(e)ine concentration in macrophages and on the capacity to release cysteine. Biol. Chem. Hoppe-Seyler 370:109 (1989).
8. H. Gmünder, H.-P. Eck, and W. Dröge. Low membrane transport activity for cystine in resting and mitogenically stimulated human lymphocyte preparations and human T cell clones. Eur. J. Biochem. 201:113 (1991).
9. W. Dröge, Krishna K. Murthy, C. Stahl-Hennig, S. Hartung, R. Plesker, S. Rouse, E. Peterhans, R. Kinscherf, T. Fischbach, and H.-P. Eck. Plasma amino acid dysregulation after lentiviral infection. AIDS Res. and Human Retroviruses 9:807-809 (1993).

10. H.-P. Eck, C. Stahl-Hennig, G. Hunsmann, and W. Dröge. Metabolic disorder as an early consequence of simian immunodeficiency virus infection in rhesus macaques. Lancet 338:346 (1991).
11. W. Dröge, H.-P. Eck, M. Betzler, P. Drings, and W. Ebert. Plasma glutamate concentration and lymphocyte activity. J. Cancer Res. Clin. Oncol. 114:124 (1988).
12. H.-P. Eck, P. Drings, and W. Dröge. Plasma levels, lymphocyte reactivity and death rate in patients with bronchial carcinoma. J. Cancer Res. Clin. Oncol., 115:571 (1989).
13. J.-S. Lim, H.-P. Eck, H. Gmünder, and W. Dröge. Expression of increased immunogenicity by thiol-releasing tumor variants. Cell. Immunol. 140:345-356 (1992).
14. S. Mihm, J. Ennen, U. Pessara, R. Kurth, and W. Dröge. Inhibition of HIV-1 replication and NF-κB activity by cysteine and cysteine derivatives. AIDS 5:497-503 (1991).
15. S. Mihm, D. Galter, and W. Dröge. Modulation of transcription factor NFκB activity by intracellular glutathione levels and by variations of the extracellular cysteine supply. FASEB J. provisionally accepted.
16. D. Galter, S. Mihm, and W. Dröge. Distinct effects of glutathione disulphide on the transcription factors NFκB and AP-1. Eur. J. Biochem. provisionally accepted.
17. R. Kinscherf, T. Fischbach, S. Mihm, S. Roth, E. Hohenhaus-Sievert, C. Weiss, L. Edler, P. Bärtsch, and W. Dröge. Effect of glutathione depletion and oral N-acetyl-cysteine treatment on $CD4^+$ and $CD8^+$ cells. FASEB J., in press (1994).
18. H. Gmünder, H.-P. Eck, B. Benninghoff, S. Roth, and W. Dröge. Macrophages regulate intracellular glutathione levels of lymphocytes. Evidence for an immunoregulatory role of cysteine. Cell. Immunol. 129:32 (1990).
19. H. Gmünder and W. Dröge. Differential effects of glutathione depletion on T cell subsets. Cell. Immunol. 138:229-237 (1991).
20. H. Gmünder, S. Roth, H.-P. Eck, H. Gallas, S. Mihm, and W. Dröge. Interleukin-2 mRNA expression, lymphokine production and DNA synthesis in glutathione depleted T cells. Cell. Immunol. 130:520 (1990).
21. S. Roth, H. Gmünder, and W. Dröge. Regulation of intracellular glutathione levels and lymphocyte functions by lactate. Cell. Immunol. 136:95-104 (1991).
22. S. Roth, and W. Dröge. Regulation of T-cell growth factor (TCGF) production by hydrogen peroxide. Cell. Immunol. 108:417 (1987).

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# NEOPTERIN AND HIV INFECTION

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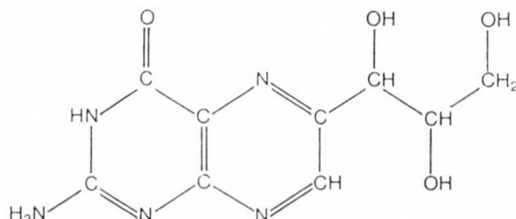


**N E O P T E R I N**
D-erythro-6-tri-hydroxypropyl pterin

**Neopterin:** In vitro, large amounts of neopterin are released from human macrophages on stimulation with interferon-γ. In vivo, neopterin concentrations sensitively indicate stimulated cellular immunity, and increased neopterin concentrations were observed during rejection episodes in allograft recipients, during acute virus infections, in autoimmune diseases and in certain types of cancer. Neopterin concentrations correlate to the extent and the activity of the disease and bear prognostic information. Besides its value as a diagnostic indicator recent studies show that neopterin may play a role within the cytotoxic repertoir of activated macrophages. Neopterin was found to enhance radical-mediated effector mechanims such as hydrogen peroxide- and chloramine-T-mediated cytotoxicity.

**Neopterin Measurements:** Measurement of neopterin is usually performed in serum and/or urine of patients. For urine determinations early morning urine samples are used, and urinary neopterin concentrations are related to creatinine levels.

Urine measurements are usually performed by HPLC. For serum determinations immunoassays are commonly used. These tests are also suited for measurements of cerebrospinal fluid (CSF). Specimens for neopterin measurements must not be exposed to direct sunlight irradiation. Repeated thawing and freezing of samples has to be avoided which is particularly important when retrospective analyses are performed.

## NEOPTERIN IN HIV INFECTION

**Acute HIV infection:** During acute HIV infection neopterin concentrations in serum and urine rise rapidly. Thereafter neopterin levels start to decline before antibody seroconversion becomes detectable. Similar observations were made also during other acute virus infections such as cytomegalovirus (CMV) infection. However in contrast to CMV, neopterin levels usually do not fully normalize in the case of HIV, and approximately 80% of HIV seropositive individuals continuously remain with elevated neopterin levels even in the asymptomatic phase of infection indicating chronic virus production at a low level.

**Stage of HIV infection:** During the course of HIV infection neopterin concentrations rise, almost all patients with AIDS-related complex or AIDS presenting with increased levels. CD4+ T-cell counts correlate inversely to neopterin concentrations, and significant correlations exist between neopterin concentrations in serum and urine and the CDC staging system as well as the Walter Reed staging classification.

**Predictive value:** In homosexual men, hemophilia patients, intravenous drug users and children with HIV infection, neopterin concentrations significantly predict progression of the disease. Patients with higher neopterin concentrations are more likely to progress to AIDS or HIV-related death than those with lower levels. This is true in early and late stages of HIV infection. Further studies show that the predictive value of neopterin is partly independent

from CD4+ T-cell counts, thus, neopterin values are joint predictors of outcome in addition to CD4+ T-cell counts. It appears that urine neopterin concentrations are superior to serum mesurements.

**Zidovudine therapy:** Therapy with ziodovudine induces significant decline of neopterin concentrations in serum and urine. The decline of neopterin concentrations in most cases becomes significant after approximately 5 days from start of therapy and reaches a plateau thereafter. On average the decline of neopterin is more long-lasting than the increase of CD4+ T-cell counts. Pretherapeutic neopterin concentrations in patients significantly predict outcome during treatment with zidovudine.

**Cerebrospinal fluid neopterin concentrations:** Intrathecal production of neopterin can be demonstrated in patients with HIV infection preferentially in those with HIV-associated dementia. In addition, neopterin in CSF correlates with severity of AIDS-dementia complex. Antiretroviral therapy leads to a decline of CSF neopterin correlating to improvement of the severity of AIDS-dementia complex in patients.

**Virus isolation:** Patients with HIV infection and positive virus isolation in culture have higher neopterin concentrations than culture-negative patients. Moreover, a correlation exists between the replicative capacity of virus isolates and neopterin concentrations: higher neopterin concentrations are observed preferentially in patients whose isolates are classified qualitatively as rapidly growing isolates with high replicative capacity (rapid/high according to B.Asjö and coworkers). These observations were made in individuals with early stages of HIV infection.

**T-cell proliferative response:** Loss of T-cell functional response is most expressed in progressed HIV infection but it may occur also in asymptomatic patients. Particularly in the patients with diminished T-cell function some-

times extremely high neopterin concentrations can be observed. Moreover, there exists an inverse correlation between loss of T-cell responsiveness in vitro and the concentrations of neopterin in patients. The data indicate that chronic activation of T cells is associated with loss of T-cell function.
Drs.Lie and Janeway have shown that interferon- plays a critical role in induced cell death (apoptosis). It appears that the status of chronic immune activation is inevitably involved in apoptosis and induction of tolerance and anergy. Pro-oxidants and Ca(2+)-stimuli are critical to induce metabolic pathways leading to apoptosis. Interestingly, neopterin not only is able to enhance radical-mediated effector mechanisms, but it also induces influx of Ca(2+) into monocytic cells in vitro. Thus neopterin may even contribute to apoptosis and T-cell anergy in patients with HIV infection.

**Neopterin compared to other parameters of immune activation:** In HIV infection, as in other diseases, neopterin concentrations correlate to serum interferon-$\gamma$ levels. Similar strength of correlation is observed between neopterin and other parameters of immune activation such as soluble tumor necrosis factor receptors and ß2-microglobulin. However, different behavior of parameters can be found in distinct risk groups for HIV exposure, e.g., no predictive value of ß2-microglobulin appears to exist in IVDUs. This observation further supports the view that immune activation plays an important role in the pathogenesis of HIV infection. In fact, also counts of activated CD4+ and CD8+ T-cells correlate to neopterin concentrations indicating that activated T cells are the source of enhanced endogenous interferon-$\gamma$ in patients with HIV infection. Similarly, mRNA expression of interferon-$\gamma$ is enhanced in T cells of patients with HIV infection.

**HIV pathogenesis:** Recently it has been shown that interferon-$\gamma$, besides other cytokines, is able to induce HIV propagation in monocytic cell lines. Thus, immune activation may play an

important role in HIV pathogenesis leading to HIV replication in infected individuals. The close relationship between neopterin concentrations and the course of HIV infection may suggest an even more direct involvement of neopterin in HIV pathogenesis. Reactive oxygen intermediates and radicals appear to be crucial to induce HIV replication via activation of the nuclear factor kB (NF-kB). Such oxygen free-radicals are common effector molecules released during the oxidative burst of activated macrophages. As mentioned above, neopterin is able to amplify radical-mediated effects in target cells. It is conceivable that neopterin also may enhance radical-mediated replication of HIV.


Fuchs D, et al. Neopterin as a marker for activated cell-mediated immunity: Application in HIV infection. Immunol Today 1988;9:150

Liu Y, Janeway C Jr. Interferon gamma plays a critical role in induced cell death of effector cells: a possible third mechanism of self-tolerance. J Exp Med 1990;172:1735

Zangerle R, et al. Markers for disease progression in intravenous drug users infected with HIV-1. AIDS 1991;5:985

Wachter H, et al. Neopterin - Biochemistry, Methods, Clinical Application. W.deGruyter 1992

Biswas P, et al. Interferon-gamma induces the expression of human immunodeficiency virus in persistently infected promonocytic cells (U1) and redirects the production of virions to intracytoplasmic vacuoles in phorbol myristate acetate-differentiated U1 cells. J Exp Med 1992;176:739

Fuchs D, et al. Neopterin, biochemistry and clinical use as a marker for cellular immune reactions. Int Arch Allergy Immunol 1993;101:1

Fan J, Bass HZ, Fahey JL. Elevated IFN-gamma and decreased IL-2 gene expression are asscoaited with HIV infection. J Immunol 1993;151:5031

Fuchs D, et al. Soluble tumor necrosis factor receptors and immune activation in human immunodeficiency virus infection. In: Molecular Biology of Hematopoiesis, vol.3, Intercept Ltd. PO Box 716, Andover, Hampshire, SP10 1YG,UK (in press)

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# COMPETITION OF COMPLEMENT PROTEINS AND SPECIFIC ANTIBODIES FOR BINDING TO HIV-1 ENVELOPE ANTIGENS

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The ability to activate the classical complement pathway is an inherent property of the transmembrane HIV envelope protein, gp41 (1). Previously we have demonstrated that the capacity of gp41 to bind to specific antibody markedly decreased after incubation in normal human serum. The inhibition was proved to be mediated through the classical complement pathway activation (2).
The same phenomenon, complement-mediated inhibition of gp41-antibody interaction was observed in a reverse system, too: rgp41-coated ELISA plates were incubated with mixtures of human polyclonal anti-gp41 antibody and fresh pooled normal human serum (NHS). A marked decrease in the amounts of the antibody fixed to the plates was observed as compared to mixtures containing the same amounts of antibodies in buffer. Similar findings were obtained in the case of plates coated with synthetic peptides corresponding to the immunodominant region of gp41. Purified C1q also strongly inhibited antibody binding to solid phase gp41 from C1q-antibody mixtures (3).
Due to the inherent complement activating capacity of gp41, a well measurable C1q, C4b and C3b binding to the rgp41-coated plates occurred from NHS with no antibodies. When, however, C1q, C4b, and C3b fixation from the antibody-NHS mixtures was measured, it was found that at higher concentrations, anti-gp41 antibodies significantly increased fixation of these proteins to rgp41 .

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Similar observations were made by 3 human monoclonal antibody preparations. These antibodies - designated as 181-D, 240-D, 246-D - were kindly provided by Dr. Susan Zolla-Pazner, Veteran Administration Center, New York). The polyclonal antibody preparation was used as control. Again different dilutions were prepared from the antibodies in buffer and NHS, the antibody-NHS mixtures were incubated in ELISA plates coated with gp41, and after washing, the amounts of fixed antibodies and complement proteins were estimated.

An almost complete inhibition of antibody binding to the ELISA plates was observed as compared to the buffer control in the case of all the four antibody preparations when they were added to solid phase gp41 in the presence of NHS or purified C1q. No significant differences were found between the 3 monoclonals (4).

When complement protein fixation was measured from the same antibody-NHS mixtures, only the monoclonal designated 246-D activated the complement since C3 and C4 fixation exceeding the control (NHS with no antibody) was observed only with that antibody and to a similar extent with the polyclonal human antibody. In the case of C1q binding, the findings were similar but not identical. Slightly more C1q was fixed to solid phase gp41 when 181-D and 240-D were present in the NHS, as compared to the only NHS control. The effect of 246-D (similarly to that of polyclonal) was, however, markedly higher.

These results indicate that the complement proteins, especially C1q, and the specific antibodies compete for a region corresponding to or close to the so-called immumodominant epitope of gp41. Previous data (summarized in ref. 1) also indicate that the site(s) responsible for complement activation, as well as C1q and C3b fixation can be localized at that region of the transmembrane HIV protein. The mechanism of the complement-antibody competetion is summarized as a hypothesis (see next page). If the hypothesis is correct, the end-result of HIV-complement-antibody interaction can be formation of HIV-com

plement complexes which do not contain significant amounts of gp41-antibodies (Similar experiments with different gp120 preparations are under-way in our laboratories). Interstingly enough, Joling et al. (5) demonstrated that binding of HIV-1 to follicular dendritic cells in vitro is complement-dependent and can occur without the contribution of antibodies.

| Hypothesis: competition between specific antibodies an complement proteins for gp41 |
| --- |
| |
| COMPLEMENT PROTEINS AND ANTIBODIES COMPETE FOR THE IMMUNODOMINANT EPITOPE OF GP41, THE AFFINITY OF THE COMPLEMENT PROTEINS IS HIGHER THAN THAT OF THE ANTIBODIES |
| Step 1a. Gp41 independently of antibodies activates the CP which leads to C1q, C4b and C3b fixation to gp41 |
| Step 1b. The antibody binds to gp41, activates CP which leads to C1q, C4b and C3b fixation to gp41 |
| STEP 1A AND 1B MAY TAKE PLACE SIMULTANEOUSLY, AND BOTH CAN RESULT IN STEP 2. |
| Step 2. Binding of complement proteins, mainly that of C1q takes gp41-antibody bonds weaker and finally can eliminate antibody from the transmembrane HIV proteins |
| END STATE: VIRUS-COMPLEMENT PROTEIN COMPLEXES WITHOUT ANTIBODIES? |

In the second part of experiments, we addressed the possible relationship between in vitro complement-activation, solid phase gp41-

antibody-complement interaction and complement-dependent enhancement of HIV infection (CDEI) using the 3 human monoclonal antibodies mentioned above. Antibodies responsible for CDEI may play a significant role in the progression of HIV disease according to our recent studies (6, and Füst et al., AIDS, in press). Little is known about the mechanism of complement-dependent enhancement of HIV infection.

CDEI measurements were performed in the presence of human complement at two different dilutions: at a final dilution of 1 to 40 (used in the experiments of Robinson et al. (7), and 1 to 4 used by our group (6). The results were as follows:

1. All the 3 anti-gp41 monoclonals tested had marked enhancing effect with both final complement concentrations (1:4 and 1:40). The higher the complement concentration was, the stronger enhancing effect was observed. Enhancement was manifested not only in the quicker appearance of HIV-1 RT in the supernatants of HIV infected MT-4 cultures but in a significantly higher peak levels, too. When we compared the 3 monoclonal antibodies, no marked differences in the CDEI were observed.

2. No correlation was found between the ability of the monoclonals to enhance HIV infection and to increase the complement binding to solid phase gp41 (Table). Only the monoclonal designated 246-D activated complement, whereas marked CDEI effect was found not only with the mAb 246-D but with the mAbs 181-D and 240-D as well.

These findings indicate that the CDEI effect cannot be simply explained by an increase of complement protein binding to the HIV-virions. Other mechanisms, like the interference of Clq binding with the gp41-gp120 bonds may be assumed. Cearly further studies are necessary to explain the meechanism of the complement-dependent HIV-infection enhancement.

COMPARISON OF THE COMPLEMENT ACTIVATING AND COMPLEMENT-DEPENDENT HIV-INFECTION ENHANCING EFFECTS OF HUMAN ANTI-GP41 ANTIBODIES

| Anti-gp41 antibody prep. | Inh. of ab.binding to gp41 by NHS/C1q | Facilitation of C1q binding | C3b | Complement-dependent HIV infect. enhancement co.dilution 1:4 | 1:40 |
|---|---|---|---|---|---|
| 181-D | ++++ | + | 0 | + | ++++ |
| 240-D | ++++ | + | 0 | +++ | +++ |
| 246-D | ++++ | +++ | +++ | + | +++ |
| polyclon. | ++++ | +++ | +++ | n.t. | n.t. |

n.t.: not tested

References


1. Dierich MP, Ebenbichler Ch, Marschang P, Füst G, Thielens N, Arlaud G., Immunology Today, 14, 435, 1993.
2. El Jarrah F, Hidvégi T, Ujhelyi E, Gyapay, G, Salavecz V, Origer J-J, Hollán SR, Füst G, AIDS, 6, 1050-1051, 1992
3. Hidvégi T, Prohászka Z, Ujhelyi E, Thielens NM, Dierich MP, Hampl H, Arlaud G,.Nagy K, Füst G, Clin.exp.Immunol.,94, 490, 1993.
4. Füst G, Prohászka Z, Hidvégi T, Ujhelyi E., Tóth D.F, Thielens N. Xth International Conference on AIDS, submitted
5. Joling, P, Bakker, L.J., van Strijp J.A.G., Meerloo T, de Graaf L, Dekker MEM, Goudsmit J, Verhoef J, Schuurman H-J., J.Immunol. 150, 1065, 1993.
6. Tóth FD, Szabó B, Ujhelyi E, Pálóczi K, Horváth A, Kiss J, Bánhegyi D, Hollán SR, AIDS 5, 263, 1991
7. Robinson WE, Montefiori DC, Gillepsie DH, Mitchell WM, J.AIDS 2, 33, 1989.

# ABSTRACTS

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# EXPOSURE TO HUMAN OR SIMIAN IMMUNODEFICIENCY VIRUS WITHOUT SEROCONVERSION INDUCES ENVELOPE SPECIFIC T CELLS AND ANTIBODIES TO MHC CLASS I ANTIGENS

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*Background*: Helper T cell responses to HIV-1 envelope peptides have been demonstrated in HIV antibody negative individuals who are at risk for HIV infection. The absence of overt signs of infection in individuals continuously exposed to HIV suggest the possibility of protective immunity. Protection in animals has been associated with the development of antibodies that recognize MHC antigens. Similar antibodies have also been detected in human volunteers vaccinated with candidate HIV envelope vaccines. We investigated whether exposure to the virus without seroconversion induces both types of responses.
*Methods*: Envelope (Env) specific helper T cell responses (TH), detected as in vitro production of IL2 by peripheral blood T cells, were studied in 36 seronegative intra venuous drug users (IVDU) and 5 macaques inoculated with various dilutions of SIV. MHC class I antibodies were detected by a competition assay between serum antibodies and a HLA class I specific monoclonal antibody.
*Results*: Env-specific TH responses were detected in 22/34 (65%) of the IVDU tested and MHC class I antibodies in 15/34 (44%). 13/34 were positive in both tests and 13/34 were negative in both tests. 3/3 macaques inoculated with low dose virus were positive in both assays and did not seroconvert while 2/2 inoculated with high dose virus were negative in both assays and did seroconvert.
*Conclusions*: Env specific TH responses correlated with the presence of antibodies to MHC class I in individuals that remained seronegative despite repeated exposure to HIV. Infection of macaques with low doses of SIV appeared to induce the same types of responses in the absence of seroconversion.

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## COMPLEMENT AND HIV-1 INFECTION OF HUMAN CELLS

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**Objective**: To investigate the role of complement in HIV-1 infection of different types of cells independentely of the HIV specific antibodies.

**Methods**: To determine the role of complement in HIV-1 infection we incubated the HTLV-IIIB and HTLV-RF strains of HIV-1 with seronegative normal human serum under conditions which allow complement activation. The incubation of the complement-pretreated viruses was done with the human MT2 ($CR2^+$, $CD4^+$), U937 ($CR1^+$ or $CR3^+$, $CD4^+$) and RAJI ($CR2^+$, $CD4^-$) cell lines and with peripheral blood T cells. Infection of cells was assessed by measuring reverse transcriptase (RT) activity in supernatants at different times of culture.

**Results**: Incubation of different cells with complement and virus resulted in enhancement of infection of the cells when low amounts of virus were used. For MT2 cells, complement activation by viral suspensions occurred through the alternative pathway and infection with suboptimal amounts of serum-opsonized HIV-1 was suppressed by blocking the C3dg receptor (CR2, CD21) on MT2 cells with monoclonal anti-CR2 antibody and rabbit Fab'2 anti-mouse Ig antibodies. Blocking of the gp120-binding site on CD4 under similar experimental conditions had no inhibitory effect on infection of MT2 cells with opsonized-virus. For U937, RAJI cells and PBL, the mechanism of complement activation by HIV-1 will be presented as well as for the coculture of PHA-activated and HIV-infected PBL from different donors. The role of human antibodies against gp41 and gp120 in this complement pathway will be presented. The role of homologous restriction factors which protected infected cells from complement lysis will be discussed.

**Conclusion**: The complement in the absence of specific antibodies enhances infection of C3 receptor bearing cells just by the interaction of opsonized virus with the CR1, CR2, or CR3 receptors. The clinical relevances of these findings remains to be determined.

## CD4 EPITOPE MASKING BY GP120 ON LYMPHOCYTES FROM HIV-1 INFECTED PATIENTS DEMONSTRATED BY USING A MODIFIED CD4 CAPCELLIA KIT

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We recently reported a two-site ELISA immunoassay (CAPCELLIA CD4-CD8, SANOFI DIAGNOSTICS PASTEUR, *Clin Chem* 1994, 40) to measure the concentration of these molecules expressed on the surface of peripheral lymphocytes. Given the epitopic specificity of the anti-CD4 MAb (domain 1), this kit detects gp120-free CD4 molecules. Determinations with this assay in HIV-1-infected patients treated with AZT have shown a rapid increase of CD4 concentrations without a concomittant increase in the number of CD4+T cells, suggesting unmasking of CD4 molecules. To study the masking of CD4 by gp120, we developed a modified CAPCELLIA immunoassay of the CD4 molecules, using an antibody directed against a region (domain 2) on the CD4 molecule independent of the gp120-binding site. Healthy subjects taken as controls showed practically identical concentrations of the two CD4 epitopes in blood (domain 1 / domain 2 $\geq$ 0.8). The proportion of HIV-1$^+$ patients (n = 66) showing significant masking of the CD4 (ratio < 0.8) increased with the severity of the disease : 10%, 37% and 80% for categories A, B and C, respectively, of the 1993 CDC classification. Interestingly, 23% of the patients with CD4+ T-lymphocytes counts < 200 µl (n = 40) not only had a gp120-free CD4/total CD4 ratio $\geq$ 0.8 but also presented a good clinical status as indicated by a Karnofski's index $\geq$ 90%. These results strongly suggest that the modified CAPCELLIA should prove to be an important diagnostic tool giving a new approach of the clinical status not observed by conventional enumeration.

**LONGITUDINAL ANALYSIS OF THE ANTIBODY RESPONSE TO HIV-1 P17 AND P24 IN PATIENTS WITH HAEMOPHILIA**

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***Objectives***: To study the antibody response to HIV gag proteins longitudinally for up to 9 years, specific p17 and p24 IgG were titrated and affinity constants were determined. To assess their prognostic value, results were related to CD4 cell counts and clinical course of the patients.

***Patients & Methods***: 16 HIV-1+ patients with haemophilia were studied during the period 1984-1993. Following their 1993 clinical status patients can be divided into two groups; Asymptomatic: 9 patients CDCII - AIDS: 5 patients died of AIDS related diseases and 2 patients are CDCIV. Patients' sera were titrated against recombinant (r) HIV-$1_{HXB2}$ p17 and p24 in ELISA. IgG affinity constants were determined using a double isotope radioimmunoassay. Predetermined sera working dilutions were incubated with a range of 10 Antigen (Ag) concentrations containing a constant amount of labelled Ag. Affinity constants (K) were calculated: 1/bound Ag = 1/Abt $x$ 1/K $x$ 1/free Ag + 1/Abt (Abt: total antibody binding sites).

***Results***: All patients who developed AIDS had a continuous decline in p17 and p24 IgG titres from 1986. This decline preceded by 4-5 years the fall of CD4 cells to under 200/µl. Over the entire study, patients with stable or rising IgG titre remained asymptomatic. The IgG affinity values in asymptomatic patients (mean K= 25.5 $10^6$ $M^{-1}$) was higher than in patients who developed AIDS (mean K= 7.16 $10^6$ $M^{-1}$). On a longitudinal basis patients who developed AIDS lost high affinity antibodies early in the infection; in contrast asymptomatic patients maintained high affinity antibodies.

***Conclusions***: Progressive reductions in IgG titres and affinity were earlier predictors of disease onset than CD4 cell counts by several years. Failure to maintain high affinity gag specific IgG , suggests that $T_H$ cells have impaired function early on. Anti-HIV antibody affinity should be monitored to established whether a protective antibody response could be associated with high affinity antibodies.

ENHANCEMENT OF HIV-1 GP41 BINDING TO RAJI CELLS BY MITOGENS AND CYTOKINES

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Based on our finding that HIV-1 gp41 independently of CD4 can bind to several proteins (gp41 binding protein) on the human T cell line H9, B cell line Raji and monocyte cell line U937, we examined the effect of mitogens and cytokines on binding of gp41 to H9, Raji and U937 cells. Flow cytometry (FACS) analysis demonstrated that PWM and LPS, IFN-$\gamma$ and IL-6,but not Con A, IFN-$\alpha$, $\beta$ , $\omega$ , and IL-2, could increase gp41 binding to Raji cells. In controls, none of the regulators (IFN-$\alpha$, $\beta$ , $\gamma$, $\omega$, IL-2, IL-6, Con A, PWM, LPS) could modify the binding potential of H9 and U937 cells. Our data suggest that the expression of HIV-1 binding proteins is subject to regulation by PWM,LPS,IFN- and IL-6 in case of B cells, while on T cells and macrophages the binding proteins may be constitutively expressed.

ANALYSIS OF RELATION BETWEEN SYMPTOMATOLOGICAL STATUS AND IMMUNOLOGICAL MARKERS (IL-2R,TNF,CD4s and CD4 SUBSET ) IN THE COURSE OF HIV INFECTION

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Circulating lymphocyte flow cytometry analysis is the most useful and the most applied procedure to study immune modifications in the course of HIV infection and to compare these findings with other immunological markers and symptomatological status.
We followed for eight months 68 HIV positive patients (48 males and 20 females) randomized into two groups: group A) Asymptomatic subjects - group B) subjects staged in IV group (CDC classification,1987).
Inthese patients we compared some immunological markers (IL-2R soluble,TNF,CD4soluble and CD4 percentage) with symptomatological status and disease stage.
After eight months of follow-up we demonstrated a significative difference between the groups in two parameters,CD4 soluble and CD4 percentage ($p < 0.001$), either at the beginning ,either at the end of the study, while the difference of IL-2R was significative ($p < 0.001$) only at the beginning of the follow-up.
Nevertheless our data didn't reveal any significative difference,inside of the same group,between the beginning and the end of our study,except in IL-2R levels in patients randomized in group A.

# CD4 CELL COUNT AND p24 ANTIGENEMIA IN PREDICTING HIV DISEASE PROGRESSION: A COMBINED EVALUATION OF 18 MONTHS FOLLOW-UP.

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Flow cytometry is widely used in the monitorship of HIV infection to determine CD4 cells cont, considered the most significative marker of this infection. CD4 lymphocyte count or percent alone are not,however, precise markers of disease progression.
Active HIV replication seems to be revealed by p24 antigen(one of core proteins) detection in the serum of seropositive subjects. To demonstrated the use - fulness of combined evaluation of CD4 lymphocyte cells and p24 antigen level in predicting the HIV disease progression, we studied these markers in 68 HIV positive patients (48 males and 20 females) followed-up for 18 months HIV disease progression was strictly related to the CD4 entry level; twenty patients showed detectable p24 antigen at the beginning of the study. In these patients we observed a more rapid decline in CD4 cells count compared to p24 negative ones.
We demonstrated that the persistance of p24 antigen with increasing level, is correlated to progression to AIDS and to a more rapid decrease of CD4 lymphocyte population, linked to a more rapid clinical disease progression.

**HUMAN MONOCLONAL ANTIBODIES DIRECTED AGAINST THE ENVELOPE OF HIV1 FOR PASSIVE IMMUNOTHERAPY**

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**Objective**: To produce in vitro human monoclonal antibodies (HMabs) against the envelope proteins of HIV with neutralizing potential for therapeutic applications with different methods.

**Methods:** Epstein Barr virus transformation or fusion with murine myeloma of peripheral blood B lymphocytes of subjects with neutralizing antibodies or after *in vitro* primary or secondary stimulation of lymphocytes from HIV seropositive or seronegative individuals with specific envelope peptides.

Screening was done by ELISA on complete virus and with synthetic peptides. Neutralization was evaluated for free virus on MT4 or H9 cells system, and for passage of virus from cell to cell with syncytium inhibition of H9 infected cells with SUPT1. The enhancing potential were achieved with MT2 and complement and the ADCC analysed on U937 infected cells..

**Results**: 15 stable clones producing HIV specific antibodies established with the different methods will be presented with their principal characteristic features as : FACS analysis, peptide identification, neutralizing or enhancing potential, complement activation and ADCC. The synergetic potential of these HMabs will be discussed.

**Conclusion**: Human anti-HIV Mabs can be obtained with different methods. These HMabs will help us to further characterize the humoral response to HIV infection, define biologically significant determinants on HIV proteins, and may be useful in clinical applications as passive immunotherapy and in a first purpose to prevent the infection after an accidental contamination.

# HIV-1MN-DIRECTED NEUTRALIZING ANTIBODIES AND NONINFECTIOUS VIRAL ISOLATES ASSOCIATED WITH PROLONGED SURVIVAL IN ASYMPTOMATIC AFRICAN CHILDREN WITH PERINATALLY HIV-1 INFECTION

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**Objectives** : Analysis of humoral response and HIV-1 isolates in 11 out of 537 HIV-1 perinatally infected children from Rwanda after more than 10 years.
**Methods** : Neutralizing antibody titers were determined in cell-free and in cell-to-cell infection assays with different HIV-1 strains. The reactivity of the sera was assessed by ELISA against the Principal Neutralizing Domain (PND) of gp120 from different HIV-1 strains and against V3 consensus peptides (US/Europe and Africa). Viruses were isolated from PBLs of four children with high neutralizing titers and studied for their biological properties.
**Results** : Nine sera inhibited syncytia formation induced by HIV-$1_{MN}$-infected cells with titers from 40 to 320. Seven and nine sera neutralized cell-free virus infection of MT4 cells with IIIB and the African strain RII, respectively (titers from 80 and 640), while ten neutralized MN infection with higher titers (80 to 2560). Nine sera showed a strong reactivity against the PND from MN but not from IIIB, RF and Z2. They also reacted against the two consensus V3 peptides. HIV-1 isolates from PBLs of four long survivors children presented a "non-syncytium inducing" or NSI phénotype on SupT1 and MT2 target cells. Infection of PBLs with these isolates could be also neutralized by their autologous serum. A previous study demonstrated in these children a persistent production of IgM directed against envelope glycoproteins. The biological properties of the specific HIV-IgM of these sera will be presented.
**Conclusion** : The presence in the sera of a neutralizing activity against different strains of HIV-1, and of HIV-1MN V3-directed antibodies, as well as the NSI properties of the isolates, could also contribute to the prolonged survival of these children. Finally, these findings suggest the prevalence of a MN-like strain in Rwanda and the usefullness of MN-like PND sequences in the development of AIDS vaccines in this part of the world.

**LTB4 RELEASE IN CEREBROSPINAL FLUID AS A MARKER FOR CEREBRAL CRYPTOCOCCOSIS IN HIV DISEASE**

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**INTRODUCTION:** Opportunistic infections of central nervous system (CNS) are frequent in AIDS; Cryptococcus meningoencephalitis is one of the most common. This latter is characterized by unspecific and limited clinical symptoms, since usually the signs of meningeal inflammation and encephalic involvement are lacking. It often occurs late in the course of HIV infection, in a phase of severe immunosuppression, but, sometimes, it may constitute the onset of AIDS and it may occur before the appearance of serum specific antibodies.

**OBJECTIVES:** Considering the fact that the involvement of the immunocompetent cells of CNS, during HIV infection, could modify the inflammatory response against the infectious agent, we evaluated the release of an inflammatory mediator such as $LTB_4$ in cerebrospinal fluid (CSF) of AIDS patients with CNS diseases.

**METHODS:** We studied 12 AIDS patients, mean age 29, with Cryptococcus meningitis, and 12 control subjects with inflammatory and degenerative pathologies of CNS. $LTB_4$ determination was performed by competitive RIA. The diagnosis of cryptococcosis was made either by culturel or immunoenzimatic tests.

**RESULTS:** We found very low levels of $LTB_4$ in all the HIV-positive subjects (mean 60.5 pg/ml).

**CONCLUSIONS:** We deem that can exist a correlation between the shaded clinical features of Cryptococcus infection in HIV patients, and the low production of $LTB_4$ by the immunocompetent cells. The low $LTB_4$ levels could depend either on an altered capacity of the producing cells or on a reduced response of the target cells to the chemotactic stimuli of $LTB_4$; both factors lead to a reduction of the inflammatory reaction in the tissue injury following the infection by Cryptococcus.

An idiotypic network model of AIDS pathogenesis that is based on coselection (mutual positive selection) of HIV and helper T cells will be reviewed

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If HIV mutates to evade the immune response, why can't that mechanism be used by any virus or bacterium? That is, why don't all pathogens mutate rapidly to escape the immune system? An alternative AIDS pathogenesis concept is a "coselection" model in which immune recognition of a particular viral strain favours the selection of that strain, which could occur if the T cell receptor is involved in the cell infection process. Then HIV strains that are recognised by as many T cells as possible would be selected. Simultaneously, helper T cells with specificity for HIV would be stimulated and thus selected, which would in turn favour the selection of suppressor T cells with idiotypes that resemble (from the point of view of Th cell idiotypes). Then immunity against HIV could with time zero in on the "centre-pole" idiotypic determinants of suppressor T cells and contribute to the destabilization of the system seen in AIDS.
The HIV quasi-species and the suppressor T cell idiotypic centre-pole would initially not be coincident in shape space, but coselection of helper T cells and HIV variants together with coselection of helper T cells would make them more and more similar as time goes on. This process could account for the variable latency period of AIDS. Inducing immunity to class II MHC (J. Scott, L. Arthur) would perturb the network along the anti-MHC--MHC-image axis, and could substantially shift the centre-pole. If the displacement in shape space is large compared with the original distance from HIV to the centre-pole this would correspond to an increase in distance in shape space from HIV to the centre-pole. This change would lead to a decrease in the amount of complementarity between helper cell idiotypes and HIV, which in turn could result in inhibition of infection. The increase in the distance from HIV to the centre-pole could finally result in a slowing down of the pathogenic process.

SECRETED PRODUCTS OF HIV-1-SPECIFIC CYTOTOXIC T LYMPHOCYTES (CTL) STIMULATE HLA CLASS I AND INTERCELLULAR ADHESION MOLECULE 1 (ICAM-1) EXPRESSION, AND INCREASE β2 MICROGLOBULIN LEVELS IN VITRO

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Objective: We have observed that besides acting directly cytolytic HIV-1-specific CTL release a variety of cytokines. To assess further the potential role of cytokines released by these CTL we tested the ability of soluble products secreted by HIV-1-specific CTL to induce HLA class I and ICAM-1 expression and to raise β2 microglobulin (β2M) concentrations in cell culture.
Methods: Supernatants were derived from HIV-1-specific CTL incubated with autologous B-lymphoblasts presenting either the cognate HIV-1 epitope or a control peptide. A variety of cell lines and peripheral blood mononuclear cells (PBMC) were incubated with these supernatants for 24-48 hours. Similarily, cell lines or PBMC were cocultured with CTL and their targets. HLA class I and ICAM-1 expression were determined by flow cytometry analyses. β2M concentrations in the cell culture supernatants were determined by ELISA.
Results: In parallel to lysis of their cognate target HIV-1-specific CTL secrete products that stimulate HLA class I and ICAM-1 expression on cell lines and on PBMC. As few as 1000 CTL significantly induced the expression of these molecules. In addition, secreted products of HIV-1-specific CTL enhanced β2M release by PBMC and Jurkat cells. Both effects were mediated primarily by IFN-γ.
Conclusions: These observations suggest that HIV-1-specific CTL may contribute to increased HLA class I expression in infected tissue and elevated ICAM-1 and β2M concentrations in serum and cerebrospinal fluid of infected individuals. In addititon, increased HLA class I and ICAM-1 expression in tissue may facilitate additional CD8+ T lymphocyte responses including immunopathologic activity.

# COMPARATIVE STUDY ON ANTIBODIES ASSOCIATED WITH DISEASE PROGRESSION IN HIV INFECTION

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Complement-dependent enhancing antibodies and anti-Fab antibodies were measured in parallel in serum samples of 7 longitudinally tested HIV-infected patients and in sera of 15 patients with advanced AIDS. HIV-infection enhancing antibodies were determined using a CR2-carrying cell line. IgG and IgA class autoantibodies directed against human IgG-Fab fragments were measured in ELISA assays. Significant negative correlations were found between CD4+ cell counts and IgG-anti-Fab and IgA-anti-Fab antibodies. A significant positive correlation was observed between complement-dependent enhancing antibodies and IgA-anti-Fab antibodies. Our findings indicate that enhancing antibodies and autoantibodies directed against IgG-Fab are not identical.

# MUCIN-LIKE SUBSTANCE TRAPING FREE ELECTRONS IN SERA OF HIV-INFECTED PATIENTS SHOWS AFFINITY TO HIV-1 ENVELOPE AND SHARES IN HIV-MEDIATED IMMUNOSUPPRESSION

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Mucin-like domains (MLD) of human peripheral mononuclear adhesins are regularly observed in sera of HIV-infected patients while their level is higher in the patients with KS. MLD show the property of traping free electrons followed by their passage to the hydoxyl group of water with the hydroxyl anion radical formation. MLD are also capable of binding to the HIV envelope recombinant protein and apparently affect thereby the HIV-1-mediated infectivity of the MT4 established cell line. According to data obtained in vitro and in vivo MLD demonstrate the immunosuppressive activity interfering, in particular, with the phytomitogen-induced activation of normal human peripheral mononuclears and with inhibiting the NK cell antitumor cytotoxicity. All these findings may indicate that MLD are probably involved to the progression of HIV infection.

**MOTHER TO CHILD TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1: ROLE OF MATERNAL ANTIBODIES MEDIATING CELLULAR CYTOTOXICITY AND VIRAL NEUTRALIZATION**

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Mother to child transmission is an important mode of spread for human immunodeficiency virus type 1 (HIV-1). This leads to a significant proportion of infected babies born to HIV-1 infected mothers. Several evidences exist to support *in utero* HIV-1 transmission processes. However the mechanism of the transmission of the virus to the newborn is not well Known. In this view, a retrospective study of sera from mothers infected with HIV-1 was undertaken to investigate whether humoral response in infected mothers correlates with vertical transmission of the virus. Therefore, sera from infected mothers (transmitters) who had given birth to infected children and from infected mothers who delivered uninfected children (non transmitters), were analyzed for the presence of antibodies that mediate antibody dependent cellular cytotoxixity (ADCC) and virus neutralization. Titer distributions in transmitter (T) and non transmitter (NT) groups were then compared. We showed that ADCC titers serum dilution giving 10% specific lysis, T=10; NT=12) and neutralizing titers in human monocyte-derived macrophages (MDM) (T=15; NT=20) were not linked to virus transmission (P=0,73 and P=0,88 respectively). However, neutralization titers in MT-2 cell line were significantly higher in NT mothers (n=21) than in T mothers (n=14). The discrepancy between neutralization titers in MT-2 cells and in MDM leads us to increased cohort size to more precisely investigate the involvement of humoral factors in protection against HIV-1 vertical transmission.

## A Study of the Disappearing IL-2R Receptors (CD25) From Lymphocytes During Simultaneous Multi-color Immunophenotyping

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Induction of IL-2 synthesis and expression of the heteromeric IL-2 receptor (R) complex are key events in T-cell activation. CD25 molecule has been recognized as a cellular activation marker with possible association related to determination of slow or fast progressing form of the HIV disease. With the recent renewed interest in depeptidyl IV (CD26) molecules in conjunction with HIV-1 infection of CD4 T-cells, the understanding of the IL-2R's contribution is essential. It is generally accepted that simultaneous multi-color immunophenotyping is the appropriate tool to develop to study activation status of leukocytes. It is hoped that with multi-fluorescence labels, immunophenotyping will provide some insight into the biology of cellular activation events during early stages of HIV infection. For the better understanding of the biology of cellular activation markers such as CD25 and CD38, simultaneous three and four color immunophenotyping protocols are replacing the traditional single and dual-color approach which are currently still utilized in clinical flow cytometry.

In this evaluation of simultaneous three and four-color protocols with CD45FITC, CD4ECD, CD3PerCP, and CD25PE, some sever restrictions have been observed. When the monoclonal antibodies are premixed, the percentage of CD25 positive cell are reduced from an initial 32% to: 19-31%, 15-24%, and 13%, correspondingly with simultaneous two, three and four-color combinations respectively. These significant losses of surface expression are only partially due to the complex color compensation matrices.

# THE ROLE OF VIRAL PROTEASE (PR) IN THE EARLY PHASE OF HIV-1 REPLICATION: PREVENTION OF PRIMARY HIV-1 INFECTION BY INHIBITORS OF PR

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HIV replication and expression is under the control of viral regulatory proteins and the endogenous cytokine network. Recently it was reported (Riviere et al.,Nature **350**:625, 1991), that in HIV infected cells - similarly to cytokines - the activation of cellular transcription factor NF-κB is also specifically induced during the course of infection, which may be regulated by viral gene product: the HIV-1 proteinase, resulting the replication of HIV.Here we provide evidence for prevention of primary HIV-1 infection *in vitro* by inhibitors of viral PR, and for its role in the early phase of viral replication. The antiviral activity of substrate-based inhibitors of HIV PR, UK-88,947 and UK-112,812 was quantitatively determined in a single cycle of infection using the MAGI infectivity assay (Kimpton and Emerman, J.Virol. **66**:2232, 1992). Both compounds inhibited primary infection by $HIV\text{-}1_{IIIB}$ and the replication-defective HIV-gpt(HXB-2). $IC_{50}$ values were in the range of 12-15 μM and 3–4 μM respestively. The progression of HIV cDNA synthesis in the cells treated with PR inhibitor was analyzed by PCR at 4 h and 18 h post infection using four primer sets recognizing various regions of the viral genome. At 4 h cDNA was detected with all primer pairs but after 18 h the *gag* and the LTR/*gag* specific primers were unable to amplify cDNA. We conclude that PR inhibitors do not affect virus entry or initiation of reverse transcription and that the cDNA transcribed in inhibitor-treated cells is not integration competent, stable cDNA. It is likely that the target of the PR in the early phase of viral replication is the nucleocapsid (NC) protein and its cleavage may be necessary for the formation of a properly assembled cDNA-protein complex, the prentegration complex and/or its transport to the nucleus. These and other results suggest that PR and NC protein itself and/or its proteolytic fragments may have an active role in the early phase of virus replication.

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## TRANSCELLULAR TRANSACTIVATION OF HIV-1 BY HHV-6

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In vivo transactivation of HIV-1 by HHV-6 has been postulated on the bases of in vitro experiments and believed to be important in the acceleration of AIDS progression. We detected HHV-6 active replication more frequently in IVDA (107/135, 79%), than in hemophiliacs (11/35, 31%, $p < 0.001$) and in blood donors (26/145, 18% $p < 0.001$). 81% (110/135) of IVDA was positive by HIV-1 DNA PCR and, in spite of specific retroviral therapy, expressed HIV-1 in 54%; 43% (58/135) of these also expressed HHV-6 sequences evidently able to transactivate HIV-1, since they effected in a two years' period, both CD4 count and mortality.

To determin if the disregulation of humoral factors present in HIV-1 infected patients could be due to humoral factors released form HHV-6 infected cells, we infected HSB-2 with HHV-6 and/or treate them with LPS. Surnatants were also mixed to CEM-ss cells infected with a range of HIV-1 inocula to quantitate syncytia, RT and p24 antigen production in time. At different time intervals aliquots of filtered, HHV-6-free supernatants, were also analyzed for cytokines and $^{35}$-S-Met labelled proteins.

HHV-6 cytopaticity in unfiltered supernatants increased from day 10 to 15 p.i. TNF-release post HHV-6 infection was biphasical, on day 1 and 4, suppressed on day 2; it peaked at 12 h with LPS treatment. HHV-6 infection resulted in no IFN release; its production induced by LPS was restricted between day 1 and 4.

Virus free supernatants of HHV-6 infected cultures obtained between 24 to 48 h p. i. increased HIV-1 production up to 30-fold, but no discrete protein band was evident in standard polyacrilamide gels to be released concomitantly. Mediators released late during full blown HHV-6 infection decreased HIV-1 production. HIV-1 activation by HHV-6 was decreased by simultaneous LPS treatment. Supernatants from LPS treated uninfected cultures increased HIV-1 production moderately. These data suggest two possible ways of transactivation of HIV by HHV-6: a moderate and continuous one by TNF and a very efficient, but transient one by an unidentified early diffusible product of HHV-6 infected cells. □

SERUM sCD23 AND IgE LEVELS IN HIV-1 INFECTION: WHAT CORRELATION ?

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OBJECTIVE The aim of the present study was to determine the possible correlation between sCD23 and IgE levels in HIV-infected patients.

METHODS We have selected 18 HIV-positive patients,who presented the following symptoms: chronic pruritic dermatitis,hypereosinophilia and high IgE levels.The blood donors,HIV-negative, as a control group,were recruited.In all patients we have examined in duplicate solubleCD23 molecule levels by ELISA test (T Cell Diagnostics,CA,MA,USA).The sensitivity of this test was: 15 U/ml.

RESULTS sCD23 levels in 16 HIV-infected patients ranged from 200 to 840 U/ml,and a significant correlation ($r=.764$; $p<.001$) was found between sCD23 and IgE levels in their serum. In these patients CD4+ cells were extremely reduced (median 60 cells/mmc).

CONCLUSION Our limited results suggest that in HIV infection it is possible a correlation between sCD23 molecules and IgE levels and the data support the hypothesis that this hyper IgE could be at least in part induced by sCD23 molecules. It is possible that the elevated presence of CD23 expression on T cells,in spite of their paucity, may lead the second signal for the production of IgE in vivo.

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**COMPLEMENT ACTIVATION BY RECOMBINANT HIV ENVELOPE PROTEINS: COMPARISON BETWEEN GP41 AND GP120**

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The human immunodeficiency virus type 1 possesses the ability to activate the human complement system. The aim of our study was to investigate which envelope glycoprotein(s) do activate the human complement system and via which pathway. We used a self-made solid phase ELISA system. Plates were coated with recombinant, non-glycosylated gp41 (N-terminal 185 amino acids) or with different recombinant gp120 preparations with or without glycosylation. Plates were then incubated with fresh, pooled human serum (NHS) or with heat inactivated (56$^{o}$C, 30 min.) human serum (HNHS) as a control. Fixed complement proteins were detected with specific, peroxidase labeled antibodies against different complement proteins (C1q, C3, C4). For colorimetric measurements we used OPD, optical density was measured at 492 nm.
We found that both gp41 and gp120 do trigger the complement cascade but in different manner. While gp41 bound C1q, C4 and C3 components, in the case of non-glycolysated gp120 we found only C3 to fix to the plate. The results indicate that the complement activation by gp41 take place through the classical pathway, whereas gp120 triggers the alternative pathway.

**Evidence for structural and functional homology between human C1q and gp120 of HIV-I**

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Earlier we provided evidence for an interaction between C1q, subcomponent of the first complement component C1, and the outer membrane part of gp41, the TM protein of HIV-I (Ebenbichler et al. J. Exp. Med. 1991). On the other hand it is well established, that gp41 interacts with gp120, the SU protein of HIV-I. We therefore asked the question, whether structural and functional homologies exist between C1q and gp120. First, in addition to the earlier described C1q binding site, we identified one further C1q binding region in the N-terminal fusion part of gp41 (peptide P526: aa 526-538). Second, we studied the effect of gp120 on the gp41/C1q interaction. Preincubation of recombinant soluble gp41 (rsgp41; aa 539-684) or P526 with gp120 abolished binding of C1q; gp120 and C1q competed for binding sites in rsgp41 and P526. Third, a polyclonal antiserum to gp120 recognized C1q and an antiserum to C1q recognized gp120 in both ELISA and agglutination assay. These data suggest competition of gp120 and C1q for the same sites in gp41, antigenetic homology between gp120 and C1q, and raise the possibility of an autoimmune phenomenon on the basis of molecular mimicry.

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**IgA-ANTI-FAB AUTOANTIBODIES AND DISEASE PROGRESSION IN AIDS**

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We showed recently that IgA antibodies directed against the Fab portion of the IgG molecule are strikingly associated with disease stage in patients with head and neck cancer, who commonly have immune defects. Furthermore, we demonstrated that kidney graft recipients with a high pretransplant IgA-anti-Fab autoantibody activity had a significantly better graft survival rate than patients with a low pretransplant IgA-a-Fab, underlining the immuno-suppressive property of these antibodies. In this study we investigated whether IgA-a-Fab antibodies occur during HIV infection and are associated with disease progression. 130 sera of 33 HIV+ hemophiliacs with AIDS/ARC, 178 sera of 73 asymptomatic HIV+ patients, 47 sera of 38 HIV- hemophiliacs, and sera of 72 healthy controls were tested in an ELISA. AIDS patients had significantly higher IgA-a-Fab than ARC patients, asymptomatic HIV+ patients, HIV- hemophiliacs, or healthy controls ($p<0.02$, 0.0001, 0.001, and $<0.0001$, respectively). An inverse association was found between IgA-a-Fab and CD4 counts ($r=-0.4$, $p<10^{-6}$), and this was confirmed in sequential follow up studies. Moreover, IgA-a-Fab was associated with serum neopterin ($r=0.3$, $p<10^{-5}$) and, in a separate study, with complement-dependent enhancing antibodies ($r=0.4$, $p<0.005$). Sera with high titers of complement-dep. enhancing antibodies had a 3 times higher IgA-a-Fab than sera with low titers ($p<0.005$). Affinity purified a-Fab bound to rgp120-coated, uninfected CD4+ T cells. A-Fab antibodies appear in different diseases in association with a suppressed immune status and seem to reflect a disturbed immune network. A-Fab may contribute to the elimination of CD4+ T cells by crosslinking the CD4 molecule via gp120 or gp120/a-gp120 attachment with the TcR.

## TWO COUNTERACTING GROUPS OF AUTOANTIBODIES IN AIDS

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We reported recently on an association of anti-Fab autoantibodies with disease progression and loss of CD4+ T cells in HIV+ hemophilia patients (Blood 1992; 79:954). We now tested sera of the same HIV+ patients and controls for antiidiotypic activity against anti-gp120, anti-CD8, anti-CD4 monoclonal abs, subclass controls, and rCD4. A significant association was found between a-a-gp120 and a-a-CD8 or a-rCD4 activities (both: $r>0.7$, $p<10^{-6}$). Follow up studies confirmed that these activities decreased and increased in parallel during disease development. Importantly, an opposite behaviour was observed between these three activities and a-Fab autoantibodies. The highest a-rCD4 activity was found in HIV+ patients with 301-800 CD4 counts, whereas these patients had a low a-Fab activity. In patients with 301-800 CD4 counts the a-Fab/a-rCD4 ratio was $2.5 \pm 0.4$, significantly lower than in patients with <50 CD4 counts ($7 \pm 2.6$, $p<10^{-4}$). Affinity purified a-rCD4 antibodies contained a-a-gp120 activity which could be inhibited with rCD4. Affinity purified a-gp120 antibodies contained a-Fab activity and bound to MHC class II molecules. It is known that Fab binds rCD4, CD8 binds MHC I, and CD4 binds MHC II and gp120. Based on these interactions, the existence of two biologically distinct groups of molecules with similarities to MHC I (a-Fab, a-CD8, CD4) or MHC II (gp120, Fab, a-a-gp120, a-a-CD8, a-rCD4, CD8) can be hypothesized. Disturbance of the equilibrium between group I (deleterious?) and group II (protective?) autoantibodies appears to play a role in the immunopathogenesis of AIDS.

**COMPLEMENT ACTIVATION BY GP120-COATED UNINFECTED CD4+ T CELLS**

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The mechanism of CD4+ cell depletion in HIV infected patients is poorly understood. In the present study we investigated whether gp120-coated uninfected CD4+ T cells of healthy individuals can activate complement in the absence of anti-gp120 antibodies.
In flowcytometry analysis, we found that the complement proteins C4, C3d, C5, C5b-9 and properdin bind to rgp120-precoated normal CD4+ T cells of healthy individuals when incubated in autologous serum. Complement activation occurred primarily via the classical pathway; in the presence of EDTA, Mg-EGTA or C4-deficient human serum, no complement proteins were bound to rgp120-coated CD4+ cells. rgp120 which was immobilized on the surface of microtiter plates activated complement in the absence of lymphocytes. In a lymphocytotoxicity assay, addition of rabbit sera from different sources or a mouse serum to rgp120-preincubated CD4+ T cells resulted in total lysis of CD4+ T cells, which was abolished by heat inactivation or in the presence of EDTA or Mg-EGTA. No lysis was observed with autologous or homologous normal human serum.
Our data indicate that attachment of free circulating gp120 molecules to uninfected CD4+ T cells can activate complement in the absence of anti-gp120. Although not resulting in direct cell lysis, complement activation with sub-sequent opsonization may represent a mechanism for the elimination of uninfected CD4+ T cells by the reticuloendothelial system.

## INTERACTION BETWEEN THE C1 COMPLEX OF HUMAN COMPLEMENT AND HIV-1 INVOLVES THE C1q GLOBULAR HEADS AND A MAJOR SITE ON THE TRANSMEMBRANE ENVELOPE GLYCOPROTEIN gp41

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Previous studies have provided evidence for activation of the human C1 complex by HIV-1, resulting from direct interaction between C1q and the external portion of the viral transmembrane envelope protein, rsgp41. The present study was undertaken to locate more precisely, within C1q and rsgp41, the sites involved in the C1/HIV-1 interaction. Using a solid-phase binding assay, we showed that $^{125}$I-labeled C1q binding to rsgp41 was dose-dependent, saturable, and comparable to binding of C1q to IgG-ovalbumin immune complexes. The globular (GR) and , to a lesser extent, the collagen-like (CLR) regions of C1q both bound to rsgp41. In contrast, the GR inhibited the C1q/rsgp41 interaction, whereas the CLR did not. A series of peptides covering the putative C1q binding site on gp41 (positions 590-613 of gp160) were synthesized and used as competitors in the C1q-rsgp41 binding assay. Peptide 601-613 (GIWGCSGKLICTT) inhibited C1q binding the most efficiently, with 50% inhibition at a concentration of 100 $\mu$M. This peptide also inhibited binding of C1q to rsgp36, the protein of HIV-2 homologous to rsgp41. The inhibitory effect of this peptide was dependent in part on the presence of the S-S bridge normally connecting Cys 605 to Cys 611, as reduction of this bond significantly reduced its efficiency. These data suggest that the C1q/HIV-1 interaction involves a site on C1q located within the globular regions, and a major site located within the immunodominant domain of HIV-1, which shares homology with the corresponding region of HIV-2.

**ALTERED PRODUCTION OF CYTOKINES MODULATING HEMOPOIESIS BY CIRCULATING CELLS OF SUBJECTS WITH HIV INFECTION**

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Introduction: We recently observed a direct, inhibitory activity on in vitro hemopoiesis by autologous T lymphocytes from HIV+ subjects. Actually, supernatants from in vitro coltures of T lymphocytes of HIV+ individuals demonstrated a decreased stimulating activity and an increased inhibitory activity on in vitro growth of hemopoietic progenitor cells. In order to better define which soluble factor(s) might be involved in such biological activity, we studied the production of cytokines with known effect on hemopoiesis from circulating mononuclear cells of HIV+ individuals.

Methods: Short-term (72h) in vitro coltures of T lymphocytes were established using standard methods with and without mitogen (PHA) from 9 HIV individuals (6 males - 3 females, age range 21-28 years) and 7 age- and sex-matched healthy volunteers (used as controls). Concentrations of IL-3, IL-6, GM-CSF, TNF-$\alpha$, TNF-$\beta$ and TNF-$\gamma$ were evaluated in supernatants (SN) using specific commercially available immuno-assays.

Results: In the absence of mitogen, concentrations (pg/ml) of both TNF-$\alpha$ and TNF-$\beta$ in SN (mean $\pm$ SD) were found to be significantly higher in HIV+ subjects than in normal controls (324$\pm$ 168 vs 62,8$\pm$56,9, $p< 0,001$ and 210$\pm$271 vs 12,5$\pm$ 10,7, $p< 0,001$, respectively). In SN from coltures of PHA-stimulated T lymphocytes GM-CSF concentrations (pg/ml) were lower in HIV+ subjects when compared to normal controls (6419.9$\pm$6522 vs 15584$\pm$ 2783.4, $p< 0,05$)

Conclusions: Our date indicate that T lymphocytes from HIV + subjects spontaneously produce increased amounts of cytokines with inhibitory effects on hemopoiesis such TNF-$\alpha$ and TNF-$\beta$, while, where stimulated, shown a decreased production of GM-CSF. These data suggest that an abnormal production of cytokines modulating the hemopoiesis by autologous lymphocytes might cooperate to the defective hemopoiesis associated with HIV infection.

# ANTIBODY-DEPENDENT ENHANCEMENT OF HIV-1 INFECTION IN HUMAN TERM SYNCYTIOTROPHOBLAST CELLS CULTURED IN VITRO

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We examined if Fc receptor-mediated antibody-dependent enhancement (FcR-ADE) of complement-mediated antibody-dependent enhancement (C'-ADE) of virus infection can contribute to increasing replication of HIV-1 in human syncytiotrophoblast (ST) cells. We found that both FcR-ADE and C'-ADE may result in enhanced virus release from HIV-1-infected ST cells. We showed that FcR-ADE of HIV-1 infection is mediated by FcRIII and other FcR(-s) belonging to undetermined Fc classes and does not require CD4 receptor, whereas C'-ADE uses both CD4 and CR2-like receptors. FcR-ADE seems to be more efficient in enhancing HIV-1 replication than C'-ADE. While FcR-ADE leads to increased internalization of HIV-1, C'-ADE does not result in enhanced endocytosis of the virus. In addition, antibodies mediating FcR-ADE are reactive with the gp120 viral envelope antigen, whereas antibodies involved in C'-ADE react with the viral transmembrane glycoprotein gp41. Our data suggest that both FcR-ADE and C'-ADE may contribute to the spread of HIV-1 from mother to the fetus.

A human monoclonal antibody against HIV-1 gp120 capable of broad neutralisation, complement activation and ADCC

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Previously we established a panel of human monoclonal antibodies against HIV-1. Out of these we identified the anti-gp120 humAb 2G12 showing broadly neutralising and syncytia inhibiting activity against divergent isolates of HIV-1. This inhibiting activity of HumAb 2G12 was found with laboratory strains of HIV-1 (IIIB, RF, SF2) as well with a panel of primary isolates of SI and NSI phenotype. No syncytia inhibition was observed when the MN strain was used as virus inoculum although 2G12 binds to MN infected cells. Testing the influence of active human complement in syncytia inhibition assays we observed that 2G12 also effectively inhibits syncytia formation with MN when active complement was added to the assay. Additional experiments revealed that 2G12 mediates a remarkable deposition of C3 to MN- and RF-infected CEM,NKR cells. 2G12 was also found to be a potent mediator of ADCC against cells infected with HIV-1 strains IIIB, MN and RF. We assume that 2G12 due to its outstanding properties is an interesting candidate for passive immunotherapy in AIDS.

INHIBITION OF NORMAL NATURAL KILLER ACTIVITY BY SERA FROM HIV+ HAEMOPHILIC PATIENTS: ACTION OF IL-2

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Deficient natural killer (NK) cell activity has been observed in haemophilic patients (He), with positive serology for the human immune deficiency virus (HIV). We previously demonstrated the inhibitory effect on normal NK activity by $HIV^+$ sera from He, which increased as the infection progressed.
Sera were obtained from $HIV^+$ He n=20 classified according to the CDC (groups III, IVA, IVC2 and AIDS), and normal controls (N) n=18. The cytotoxic assay was done using normal peripheral blood mononuclear cells (PBMC) as effector cells and $^{51}Cr$-K562 as target cells during 4 h at 37 °C. Cells were treated or not with interleukin 2 (IL-2) (18 h) before or after 4 h incubation with sera or simultaneously during 18 h.
NK function was significantly inhibited by $HIV^+$ sera both under 4 or 18 h incubation in comparison to N. Inhibition % x±S.E.: a) $HIV^+$ 4 h: 22.3±3.2; b) $HIV^+$ 18 h: 41.7±6.6; c) N 4 h: 4.2±1.1; d) N 18 h: 7.1±2.6. The results of IL-2 treated cells were the following: inhibition % x±S.E.: e) IL-2, $HIV^+$: 23.1±3.3; f) $HIV^+$, IL-2: 12.6±3.2;g) $HIV^+$ + IL-2: 26.6±7.4. (a vs. e: p=0.76; vs. f: p=0.007; b vs. g: p=0.015). The inhibitory effect of sera was similar in all the groups of patients studied. Treatment of the PBMC with sera from N controls did not modify the NK activity in any experimental group.
These results indicate that IL-2 activation of normal NK activity does not change the inhibitory effect by sera, but if IL-2 activation occurs at the same time or after sera treatment, a partial restoration on NK function is observed. Serum factors present in $HIV^+$ sera can aggravate the functional defect of NK cells in HIV infection, thus is important that a lymphokine like IL-2 could partially revert this effect.

## A SEVEN YEAR STUDY OF 200 HIV+ve DRUG USERS

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Introduction

This cohort of HIV-infected intravenous drug users all seroconverted during 1983-4. The trend in CD4+ T-lymphocyte was the main laboratory marker studied. A matched HIV -ve control cohort was studied in parallel.

Method

CD4 cells were stained using dual labelled monoclonal antibodies (Becton Dickinson),followed by red cell lysis and flow cytometric analysis (FACScan).

Results **% of Cohort**

| **CD4 Band** | **1988** | **89** | **90** | **91** | **92** | **93** | **94** |
|---|---|---|---|---|---|---|---|
| 600+/cmm | 29 | 6 | 4 | 1 | 0 | 0 | 0 |
| 400-600 | 47 | 37 | 26 | 5 | 7 | 2 | 2 |
| 200-400 | 24 | 44 | 47 | 33 | 22 | 18 | 2 |
| 100-200 | 0 | 12 | 16 | 30 | 18 | 27 | 18 |
| 0-100 | 0 | 1 | 7 | 31 | 53 | 63 | 28 |
| DEATHS | 0 | 4 | 5 | 16 | 22 | 28 | 38 |

Conclusions

The CD4+ T-lymphocyte count remains the best immunological laboratory marker for HIV disease progression.From results above we can see a steady "shift to the left"in 7 years. The control population showed no significant change. CD8+ and HLA DR+ both showed an increase until later stages of AIDS. IgA was the only significant immunoglobulin to be elevated. Beta 2 microglobulin was also found to be raised and used as a disease progression marker. The cohort remains under immunological surveilance.



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